AD_____

Award Number: W81XWH-10-2-0189

TITLE: Pulmonary Stress Induced by Hyperthermia: Role of Airway Sensory Nerves

PRINCIPAL INVESTIGATOR: Lu-Yuan Lee, Ph.D.

CONTRACTING ORGANIZATION: University of Kentucky
Lexington, KY 40506-0057

REPORT DATE: December 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

□X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
December 2014	Annual	30Sep2013 – 29Sep2014

4. TITLE AND SUBTITLE

Pulmonary Stress Induced by Hyperthermia: Role of Airway Sensory Nerves

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0189

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Lu-Yuan Lee, PhD

email: lylee@uky.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Kentucky
201 Kinkead Hall
Lexington, KY 40506-0001

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command,
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Based upon the results obtained from these studies, we can draw the following conclusions: 1) Airway hyperresponsiveness developed in Ova-sensitized mice was less pronounced in TRPV1-null mice, indicating an important role of TRPV1. 2) An increase in airway temperature within the normal physiological range triggered bronchoconstriction in sensitized rats, but not in control rats. Chronic airway inflammation in sensitized animals is likely a major contributing factor in causing this response. 3) transient increase in airway resistance was generated immediately after hyperventilation with warm humid air in patients with mild asthma, but the same warm humid air challenge failed to cause any bronchoconstriction in healthy subjects. Furthermore, this bronchoconstriction is likely generated by the increase in airway temperature because hyperventilation with humidified air at room temperature did not generate any change in airway resistance in the same patients. These studies, once completed, should provide important and novel information for: 1) documenting the pulmonary stresses induced by hyperthermia in healthy individuals and in patients with sensitized airways; 2) understanding the mechanism underlying the hyperthermia-induced pulmonary dysfunction; and 3) detecting the susceptibility to heat stress in soldiers with underestimated or overlooked airway hypersensitivity such as in airway allergy or mild asthma.

15. SUBJECT TERMS

Hyperthermia, asthma, airway constriction, cough, dyspnea

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			
U	U	U	UU	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION

The primary hypothesis of this TATRC project is that the expression of the transient receptor potential vanilloid type 1 (TRPV1) channel is up-regulated in the airway mucosa of patients with mild asthma, allergic rhinitis and upper respiratory infection, which makes these patients more susceptible to the bronchoconstriction and other respiratory dysfunctions induced by thermal stress.

There are three specific aims for the first no-cost-extension year of this translational project: 1) To complete the study the role of TRPV1 and tachykinins in the augmented airway response to hyperthermia in an animal model of asthma. 2) To report the results obtained from our completed study of thermal stress generated various airway dysfunctions (airway constriction, cough, etc.) in patients with allergic rhinitis. 3) To determine if thermal stress generated various airway dysfunctions in patients with laryngopharyngeal reflux. 4) To determine if thermal stress generated various airway dysfunctions in patients recovering from upper airway infection.

BODY

In the last twelve months, we have made major research progresses in this project. Two study series have been completed, and six papers have been published (see Publication List in page 11); reprints of these full papers are submitted in the Appendix, and therefore only short summaries are presented here:

Task 1-3: To determine the role of TRPV1 and tachykinins in the augmented airway response to hyperthermia in an animal model of asthma (ovalbumin-sensitized Brown Norway rats).

Summary: Our recent study demonstrated that hyperventilation of humidified warm air induces bronchoconstriction in allergen-sensitized rats. However, it is not known whether the increase in airway resistance was generated by smooth muscle contraction or extravasation in the airways. (Hsu et al., J. Appl. Physiol. 2013). Sustained stimulation of TRPV1-expressing C-fiber sensory fibers innervating rat airways is known to trigger the release of tachykinins (substance P and NKA) from these nerve endings that can cause extravasation of macromolecules and mucosal edema in the airways, leading to an increase in airway resistance. This study was designed to test this hypothesis in an animal model of allergic asthma (young Brown-Norway rats actively sensitized by inhalation of ovalbumin (Ova) aerosol for 3 weeks). These rats were divided into two groups: control and sensitized groups. Evans Blue dye was intravenously injected into the femoral vein 10 min before HWA challenge. Ten min following the HWA challenge, the lungs were removed and then separated into major airways and lung parenchyma. The magnitude of airway extravasation was quantified by measuring the amount of Evans Blue dye bound to the macromolecules (proteins) that leaked into the extravascular space, and its concentration was measured by the optical density using a spectrophotometer.

Our results obtained in this study can be summarized as follows: 1) Hyperventilation of humidified warm air induced airway extravasation in Ova-sensitized rats. 2) The airway

extravasation can be prevented by pretreatment with the selective antagonist of NK-1 receptors. Based upon these results, we concluded that the endogenous release of tachykinins is responsible for the airway extravasation induced by increase in airway temperature, which is mainly mediated through activation of NK-1 receptors.

Tasks 2-1, 2-3 & 3-3: Breathing hot humid air induces airway irritation and cough in patients with allergic rhinitis

Results obtained from this study series have been published in the journal **Respiratory Physiology and Neurobiology** (reprint is submitted in the Apppendix).

Summary: TRPV1 is a ligand-gated cation channel activated by capsaicin, protons and heat; and abundantly expressed in sensory C-fibres innervating the respiratory tract. Hyperventilation of humidified hot air (HA) results in reflexive bronchospasm and cough in asthmatics, probably caused by activation of TRPV1-expressing sensory nerves that are sensitized by chronic inflammation. In this study we tested the hypothesis that chronic inflammation sensitizes the TRPV1-expressing nerves innervating the upper airways of allergic rhinitis (AR) patients.

Cough frequency, airway resistance (R_{aw}) and spirometry test were measured before, during and after isocapnic hyperventilation (40% of MVV) of both humidified HA (49 °C) and room air (21 °C) (RA) for 4 minutes, and compared between AR patients and healthy subjects.

Our results obtained in this study can be summarized as follows: **i**n AR patients, cough frequency increased from 0.10 ± 0.07 at baseline to 2.37 ± 0.73 during, and 1.80 ± 0.79 (coughs/min) after HA challenge ($p<0.01$, n=7), but not after RA challenge. In contrast, neither HA nor RA had any significant tussive effect in healthy subjects (n=6). After HA challenge, the AR patients also expressed more respiratory discomfort measured by the hand grip dynamometry ($p<0.05$), but their R_{aw} did not increase. In comparison, the peak responses of cough frequency and handgrip signal to the HA challenge were significantly higher in AR patients than that in healthy subjects, but these responses were not different between these two groups after the RA challenge. Based upon these results, we concluded that HA hyperventilation triggered vigorous cough response and respiratory discomfort in AR patients indicating the involvement of airway sensory nerves innervating the upper airways. These responses may limit the exercise tolerance of these patients in hot humid environments.

Tasks 2-4, & 3-4: Cough response and vocal cord adduction is triggered by hyperventilation of humid hot air in patients with laryngopharyngeal reflux

Summary 4: Laryngopharyngeal reflux (LPR) has been identified as one of the most common diseases associated with chronic non-productive cough. Expression of TRPV1 has been recently reported in the human larynx. This study was therefore designed to test the hypothesis that chronic inflammation induces over-expression of TRPV1 in laryngeal C-fiber afferents in LPR

patients, and breathing hot humidified hot air (HA) can activate TRPV1 and elicit cough in these patients.

An increase in the frequency of the vocal cord adduction motion is known to be a reliable indication of the upper airway irritation. We have previously requested and received the approval of adding a procedure to record the vocal cord adductor motion using a flexible nasopharyngoscope and a video camera recorder during the hot air challenge in the patients with LPR in our ongoing study. However, our co-investigator, Dr. Deanne Roberts, who had the experience in performing this measurement left the University of Kentucky in June 2013. Fortunately, Brian Hixson, M.D., an otolaryngologist, has joined our team as a co-investigator and replaced Dr. Roberts. Dr. Hixon is well trained and experienced in the clinical study of vocal cord function in LPR patients, and will help us in recruiting LPR patients for the study. Documents of Dr. Hixon's biographical sketch, his letter of commitment to participate in this study, the approval letter from the University of Kentucky IRB and the approved new consent form were submitted to HRPO for approval on November 19, 2014. We will resume this study as soon as we receive the approval from the HRPO as well as the approval of our second no-cost-extension.

Our study protocols and results obtained in this study (including those collected before Dr. Roberts left) can be summarized as follows: airway resistance (Raw), spirometry indices, numbers of coughs and vocal cord adduction were recorded prior, during and after the HA challenge in 3 patients (1 male, 2 female) with LPR and 3 healthy subjects (1 male, 3 female). Isocapnic hyperventilation (40% maximum voluntary ventilation) of both humidified HA (49°C) and humidified room air (21°C) (RA) for 4 minutes were performed in separate sessions of random order in the same subject. The HA challenge consistently triggered cough in the LPR patients. Number of coughs increased from no coughs at baseline to 9.65 ± 7.55 during and to 1.02 ± 0.23 (coughs/min) after the HA challenge ($p<0.05$, $n=3$). The HA challenge did not cause any significant tussive effect in the healthy subjects. Further, hyperventilation of RA did not generate any significant change in number of coughs in either LPR or the healthy subjects. In addition, airway resistance did not significantly increase after the HA in LPR patients: Raw = 1.54 ± 0.19 cmH2O/L/sec at baseline; peak Raw = 1.83 ± 0.16 cmH2O/L/sec after HA challenge ($p>0.05$, $n=3$). In LPR patients the number of vocal cord adduction also increased: 0.25 ± 0.5 episodes at baseline; 7.88 ± 3.8 episodes with HA challenge. We will continue the study on four additional LPR patients and four additional matching healthy subjects

We believe this new study protocol will yield novel and important clinical information, and bring new insight into how the thermal stress alters the airway function in the LPR patients.

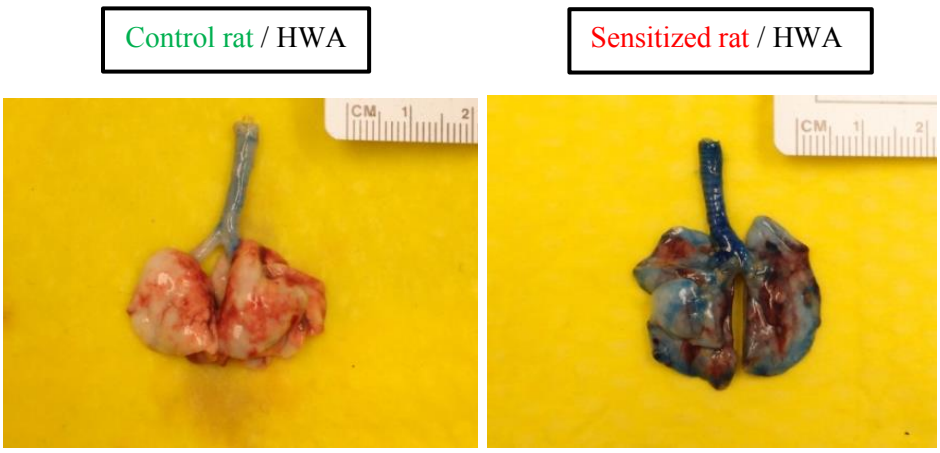

Fig. 1. Airway extravasation induced by hyperventilation with humidified warm air (HWA). Experimental photos of isolated lungs obtained from control (*left panel*; 285 g) and ovalbumin-sensitized (*right panel*; 260 g) rats. The level of blue color exhibited the content of Evans Blue dye.

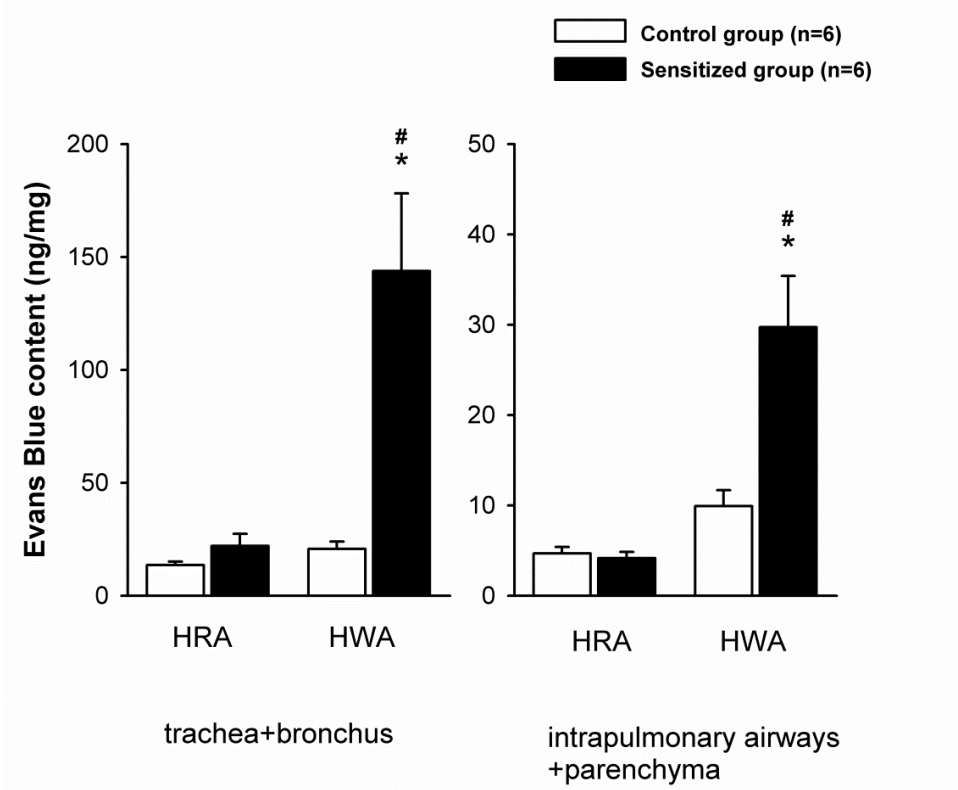

Fig. 2. Comparison of the responses of airway extravasation to hyperventilation with HWA and humidified room air (HRA) in control (open bars) and ovalbumin-sensitized (filled bars) rats. The responses of airway extravasation were calculated by Evans Blue content (ng/mg). The Evans Blue contents were obtained from main airway (trachea and bronchus; *left panel*) and small airway (intrapulmonary airway and parenchyma; *right panel*). Data are means ± SEM for 6 rats. *, significantly different from control group ($P < 0.05$); #, significantly difference between corresponding data of HWA and HRA challenges ($P < 0.05$).

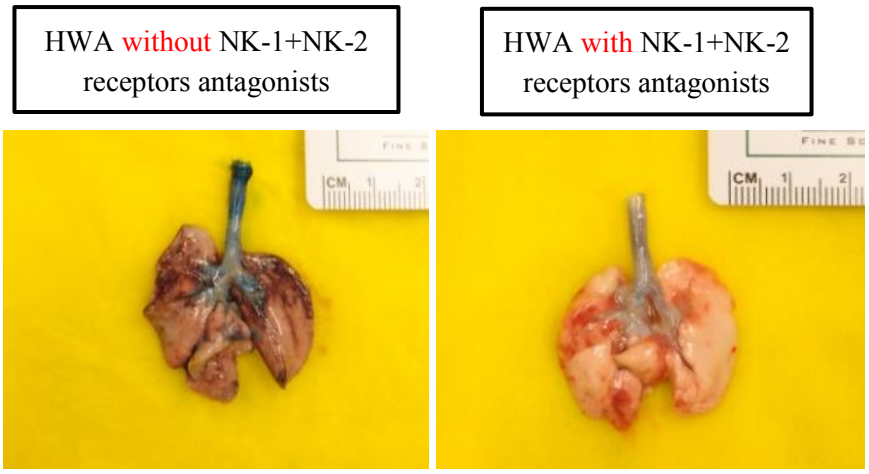

Fig. 3. Effect of pretreatment with selective antagonists of NK-1 and NK-2 receptors on the airway extravasation induced by hyperventilation with HWA. Experimental photos of isolated lungs obtained from HWA without (*left panel*; 260 g) and with (*right panel*; 255 g) pre-treatments of NK-1 (L-732138; 6 mg/kg) and NK-2 antagonists (SR-48968; 1 mg/kg) in ovalbumin-sensitized rats. The level of blue color exhibited the content of Evans Blue dye.

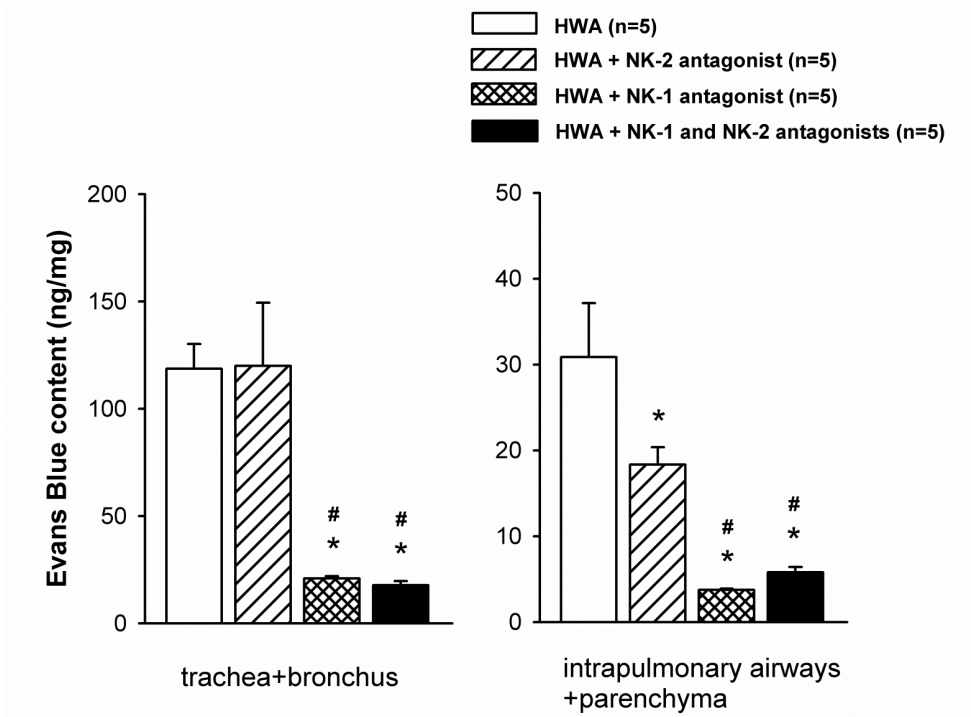

Fig. 4. Comparison of the responses of airway extravasation to HWA before (open bars) and after pretreatment with NK-2 alone (hatched bars), NK-1 (double-hatched bars) alone, and a combination of NK-1 and NK-2 antagonists (filled bars). The Evans Blue contents (ng/mg) were obtained from main airway (trachea and bronchus; *left panel*) and small airway (intrapulmonary airway and parenchyma; *right panel*). The doses of NK-2 (SR-48968) and NK-1 antagonists (L-732138) were 1 mg/kg and 6 mg/kg, respectively. Data are means ± SEM for 5 rats. *, significantly different from HWA group ($P < 0.05$); #, significantly different from HWA+NK-2 antagonist group ($P < 0.05$).

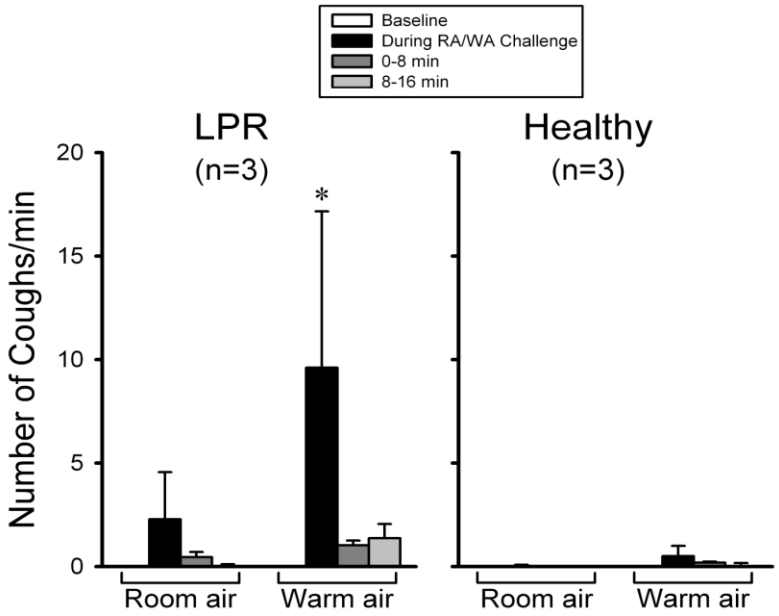

Fig. 5. Group data showing a comparison of the cough responses to hyperventilation of humidified room air and hot air in LPR patients (*left panel*; n=3) and healthy subjects (*right panel*; n=3). Cough frequencies were averaged in 8-min durations before and after hyperventilation challenge in each subject. Data are means ± SEM. *, significantly different (p<0.05) from the baseline. †, significant difference (p<0.05) comparing the corresponding data between room air and hot air.

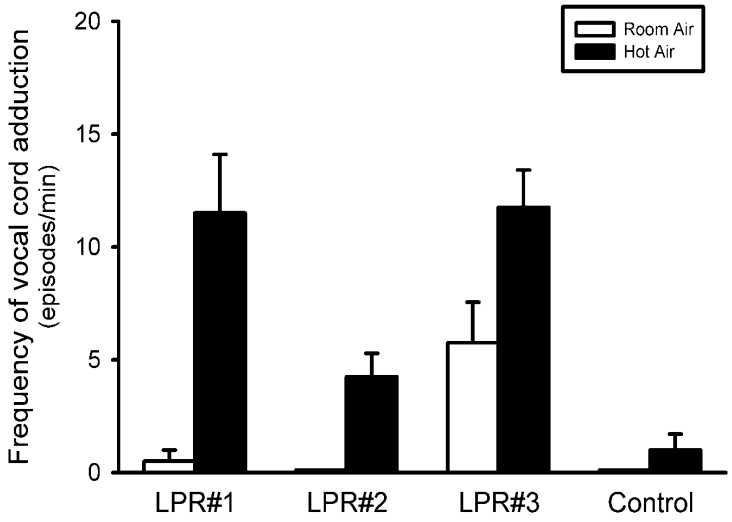

Fig. 6. Effect of hyperventilation of humidified room air and hot air on vocal cord movements recorded by a flexible fiberoptic laryngoscope in LPR patients and a healthy control subject. Vocal cord adduction greater than 50% during the inspiratory phase was considered as an indication of throat irritation, and the number of episodes during the hyperventilation challenge was measured visually by a member of the investigative team blinded to the study protocol.

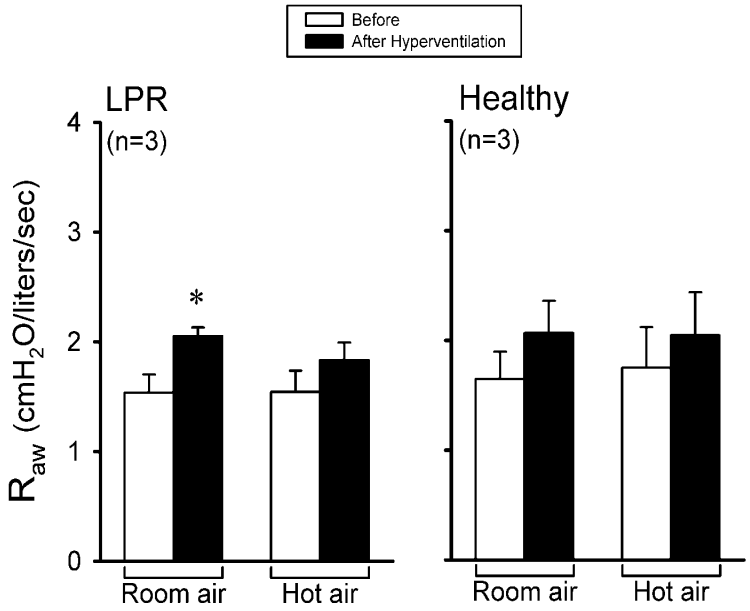

Fig. 7. Group data showing a comparison of the effect of hyperventilation of humidified room air and hot air on airway resistance (Raw) in LPR patients (*left panel*; n=3) and healthy subjects (*right panel*; n=3). Baseline (open bars) and peak (closed bars) Raw were averaged over 8 and 4 consecutive breaths before and after hyperventilation challenge, respectively, in each subject. Data are means ± SEM. *, significantly different (p<0.05) from the baseline.

KEY RESEARCH ACCOMPLISHMENTS

Our studies completed in the last twelve months have accomplished the following major milestones::

1) Establish critical information for documenting the distinct difference in these airway responses to thermal stress between healthy individuals and patients with inflammatory airway diseases.

2) Demonstrate the involvement of TRPV1-sensoty nerves in eliciting these airway response to thermal stress.

3) These new information have established important information for detecting the susceptibility to heat stress in soldiers with underestimated or overlooked airway hypersensitivity, such as in individuals with mild asthma, allergic rhinitis and laryngopharyngeal reflux.

REPORTABLE OUTCOMES

1. Publications

In the last twelve months, six papers have been published (three of them previously listed as "in revision" or "accepted for publication" in the last Annual Report), one paper is in press, and one manuscript is currently in revision:

Hsu, C.C., Y.S. Lin, R.L. Lin and L.-Y. Lee. Bronchoconstriction induced by increasing airway temperature in ovalbumin-sensitized rats: role of tachykinins. J. Appl. Physiol. 115: 688-96, 2013.

Hsu, C.C., R.L. Lin, L.-Y. Lee and Y.S. Lin. Hydrogen sulfide induces hypersensitivity of rat lung vagal neurons: role of TRPA1 receptors. Am. J. Physiol. : Reg. Int. Comp. Physiol. 305: R769-779, 2013.

Lee, L.-Y., Q. Gu, F. Xu and J.L. Hong. Acid-sensing by airway afferent nerves. Pulm. Pharmacol. Ther. 26: 491-7, 2013. (Invited review)

Lin, R.L., Y.J. Lin, M.J. Geer, R. Kryscio, and L.-Y. Lee. Pulmonary chemoreflex responses are potentiated by tumor necrosis factor alpha in mice. J. Appl. Physiol. 114:1536-43, 2013.

Lee, L.-Y., and J. Yu. Sensory nerves in lung and airways. Comprehensive Physiology (Am. Physiol. Society) 4: 287-324, 2014 (Invited review)

Khosravi, M., P.B. Collins, R.-L. Lin, D. Hayes, J.A. Smith and L.-Y. Lee. Breathing hot humid air induces airway irritation and cough in patients with allergic rhinitis. Respir. Physiol. & Neurobiol. 198: 13-19, 2014.

Lin, Y.-J., R.-L. Lin, T. Ruan, M. Khosravi and L.-Y. Lee. A synergistic effect of simultaneous TRPA1 and TRPV1 activations on vagal pulmonary C-fiber afferents. J. Appl. Physiol. PMID: 25414245 (In press; 2014)

Lin, R.-L., Y.-J. Lin, F. Xu and L.-Y. Lee. Hypersensitivity of vagal pulmonary C-fibers induced by hemorrhagic hypotension in anesthetized rats. Am. J. Physiol. – Reg. Integr. Comp. Physiol. (In revision; 2014)

2. Employment Generated by this TATRC Contract

Salaries of the employees listed below are paid in part or in full with the funds provided by this research contract during 2013-2014:

Lu-Yuan Lee, Ph.D., Principal Investigator
Mahdi Khosravi, M.D., Co-investigator
Paul B. Collins, B.S., RRT, Supervisor of Pulmonary Function Laboratory
Richard Kryscio, Ph.D., Consultant for Biostatistics
Robert Morton, Part-time Senior Research Analyst
Ruei-Lung Lin, M.S., Research Analyst (100% effort)
Alice Hsu, Ph.D., Postdoctoral Fellow (100% effort)

Reyno Tapia, Undergraduate Part-time Lab Assistant

CONCLUSIONS

Based upon the results obtained from the studies performed in our lab in the last twelve months, we can reached the following conclusions:

1) The endogenous release of tachykinins from vagal bronchopulmonary C-fibers is responsible for the airway extravasation induced by increase in airway temperature, which is mainly mediated through activation of neurokinin-1 receptors.

2) Hyperventilation of humid warm air triggered vigorous cough response and airway irritation in patients with allergic rhinitis and laryngopharyngeal reflux, indicating the involvement of the airway sensory nerves. Chronic inflammation in the upper airways may have contributed to an up-regulation of the sensitivity and/or expression of TRPV1 in these sensory nerves.

These findings have provided strong evidence in support of our hypothesis that the stress of hyperthermia exerted on the respiratory system is primarily mediated through an activation of the temperature-sensitive TRPV1 channel expressed on vagal bronchopulmonary C-fibers, and that TRPV1 expression is up-regulated in the airway mucosa of patients with chronic inflammation. However, these important observations also clearly indicated that further studies will be required to uncover the underlying mechanisms and to develop the effective preventive and therapeutic approached in order to alleviate these respiratory dysfunctions.

APPENDIX

Electronic links of of the following publications supported either in full or in part by this TATRC project are included in this Annual Progress Report below:

1. Hsu CC, Lin RL, Lee LY, Lin YS. Hydrogen sulfide induces hypersensitivity of rat capsaicin-sensitive lung vagal neurons: role of TRPA1 receptors. Am J Physiol Regul Integr Comp Physiol. 305:R769-79, 2013. http://dx.doi.org/10.1152/ajpregu.00202.2013
2. Hsu CC, Lin RL, Lin YS, Lee LY. Bronchoconstriction induced by increasing airway temperature in ovalbumin-sensitized rats: role of tachykinins. J Appl Physiol (1985). 115:688-96, 2013. http://dx.doi.org/10.1152/japplphysiol.00491.2013
3. Lee LY, Gu Q, Xu F, Hong JL. Acid-sensing by airway afferent nerves. Pulm Pharmacol Ther. 26:491-7, 2013. http://dx.doi.org/10.1016/j.pupt.2013.03.010
4. Lin RL, Lin YJ, Geer MJ, Kryscio R, Lee LY. Pulmonary chemoreflex responses are potentiated by tumor necrosis factor-alpha in mice. J Appl Physiol (1985). 114:1536-43, 2013. http://dx.doi.org/10.1152/japplphysiol.01301.2012
5. Khosravi M, Collins PB, Lin RL, Hayes D, Jr., Smith JA, Lee LY. Breathing hot humid air induces airway irritation and cough in patients with allergic rhinitis. Respir Physiol Neurobiol. 198:13-9, 2014. http://dx.doi.org/10.1016/j.resp.2014.03.013
6. Lee LY, Yu J. Sensory nerves in lung and airways. Compr Physiol. 4:287-324, 2014.

http://dx.doi.org/10.1002/cphy.c130020
7. Lin YJ, Lin RL, Ruan T, Khosravi M, Lee LY. A synergistic effect of simultaneous TRPA1 and TRPV1 activations on vagal pulmonary C-fiber afferents. (In press). J Appl Physiol (1985). 2014. http://dx.doi.org/10.1152/japplphysiol.00805.2014
8. Lin RL, Lin YJ, Xu F, Lee LY. Hypersensitivity of vagal pulmonary C-fibers induced by hemorrhagic hypotension in anesthetized rats. (In revision). Am J Physiol Regul Integr Comp Physiol. 2014.